Towards multiscale modeling: single-cell genomics and EMT regulation in cutaneous wound healing

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A Global Perspective of Cutaneous Wound Healing via Single Cell RNA-Seq:

- Wound healing of the skin represents a multifaceted repair process involving myriad cell types and cell biological processes, such as proliferation, migration, and differentiation, which are intertwined at various spatial and temporal scales.
- Here we utilize single cell RNA sequencing (scRNA-seq), in vivo murine functional assays, and stochastic multiscale modeling to investigate the cellular heterogeneity and to identify the key regulators of this regenerative process.
- scRNA-Seq was used to profile all cells present in adult murine skin during normal homeostasis and wound healing.
- Comparative analysis identified major differences in the overall cellular makeups between normal and wounded skin, uncovered previously undocumented heterogeneity within the epidermal basal populations, and revealed the enrichment of molecular signatures associated with epithelial-to-mesenchymal transition (EMT) in wound epidermal basal cells.
- Multiplexed in situ RNA detection validated the existence of multiple basal cell subsets, and pseudotemporal ordering and RNA velocity analyses suggested their novel lineage relationships and differentiation dynamics during homeostasis and wound repair.

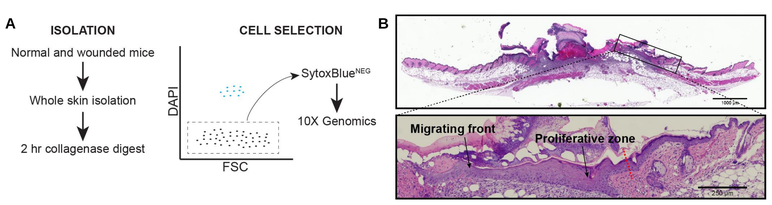

Figure 1: **Overview of single cell isolation strategy.** (A) Isolation and selection strategy to obtain single cells from wounded (WO) and un-wounded (UW) skin samples. (B) Representative H/E histology of wounded skin 4 days after wounding. Zoomed in region below highlights migrating front and proliferative zone regions of the wound epidermis.

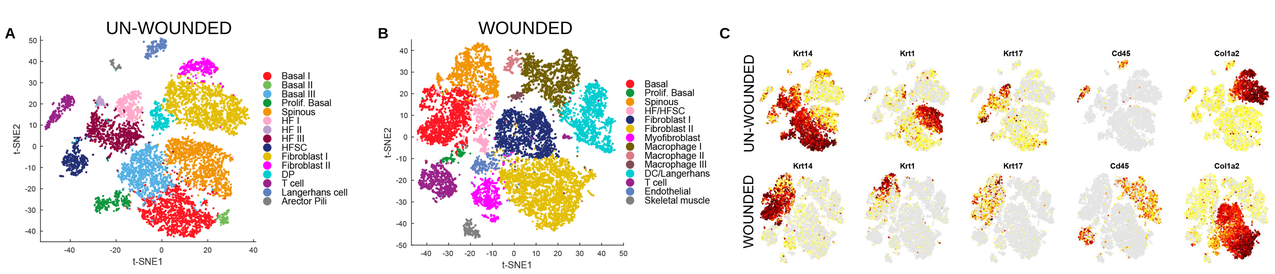

Figure 2: **scRNA-Seq reveals global changes in cellular makeup during wound healing.** (A) tSNE plot of UW sample containing total cells from two biological replicates (10,615 cells). (B) tSNE plot of WO sample containing total cells from three biological replicates (16,164 cells). (C) Feature plots from UW and WO samples highlighting expression of key marker genes for general cell types. Krt14 for epidermal basal cells and hair follicle (HF) components; Krt1 for epidermal spinous cells; Krt17 for HF components; Cd45 for immune cells; Col1a2 for fibroblasts.

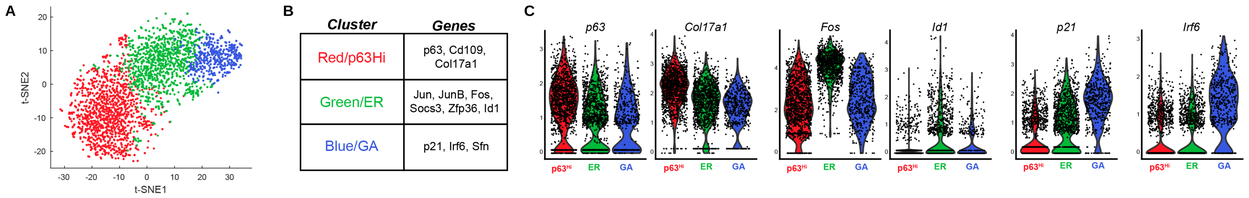

Figure 3: **Basal cell heterogeneity exists in un-wounded skin.** (A) tSNE plot of UW sample containing non-proliferative basal cells, which have unique marker genes (B). Unique marker genes lead to defining the populations as: p63Hi, early response (ER), and growth arrested (GA). (C) Violin plots for key marker genes from the three different basal populations.

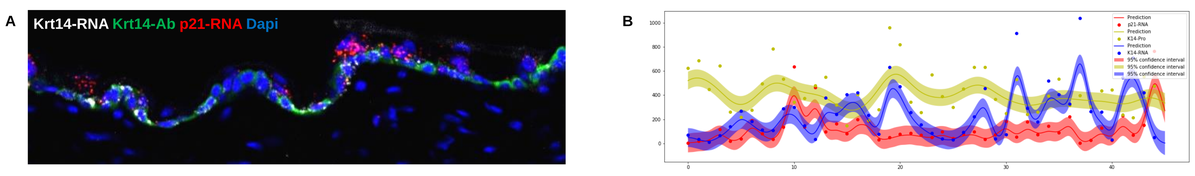

Figure 4: **In situ validation of basal cell heterogeneity in un-wounded by RNAScope.** (A) In situ analysis of marker genes using RNAScope in UW skin. Combinations of probes for RNA detection and antibodies for protein detected were utilized. (B) Quantification of Krt14-RNA, Krt14-Ab, and p21-RNA fluorescence levels (Y-axis) in individual cells (X-axis). Quantification of each cell represented by individual dots. Gaussian Process Regression utilized to generate representative line to define fluctuations in fluorescence levels.

EMT Regulation in Cutaneous Wound Healing:

- Ovol2 is a zinc finger transcriptional repressor of EMT and is expressed in the basal layer of the adult epidermis.
- *K14-Cre*-mediated deletion of *Ovol2*, leads to delayed wound healing characterized by reduced proliferation, enhanced cellular movement, but loss of directional migration of epidermal keratinocytes.
- Ovol2-deficient cells have enhanced migration and reduced directionality due to up-regulation of *Zeb1*.
- Data is published in Haensel et al., 2019 EMBO Reports.

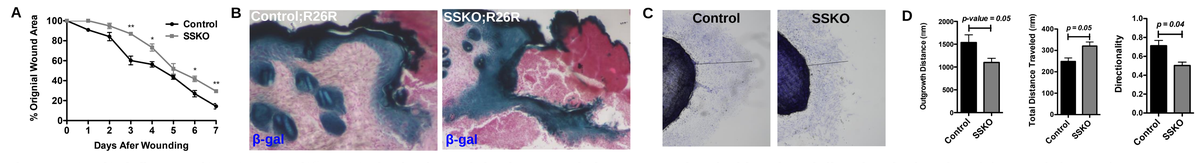

Figure 1: Ovol2-defiencent (SSKO or *Ovol2$^{f/-}$;K14Cre*) mice have defective wound closure associated with reduced directional migration. (A) Wound closure time course. (B) Lineage tracing of epidermal cells during wound healing (Control = *Ovol2$^{f/+}$;K14Cre;R26R*; SSKO = *Ovol2$^{f/-}$;K14Cre;R26R*). SSKO cells at migrating front disseminate away from epidermis. Images from wound explant assay (C) and quantification of outgrowth (D). (E) Quantification of total distance traveled and directionality of individual cells by live cell imaging for 18 hours. All experiments have n = 3 biological replicates.

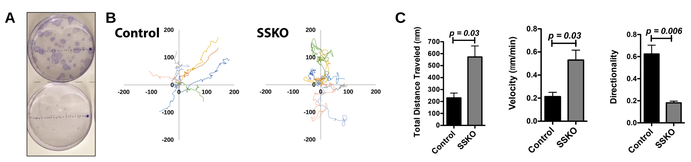

Figure 2: Ovol2-defiencent epithelial stem cells have reduced colony formation associated with enhanced migratory behaviors and reduced directionality. (A) Clonal analysis of epithelial stem cells. (B) Cell movement tracks obtained form 18 hours of live imaging from control and SSKO cells. (C) Quantification of total distance traveled, velocity, and directionality of individual cells from colonies by live cell imaging.

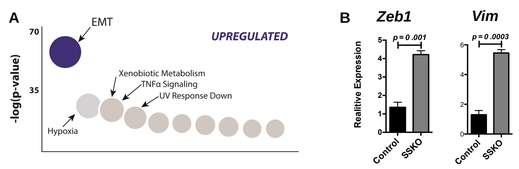

Figure 3: Loss of Ovol2 leads to up regulation of EMT genes, most notably Zeb1. (A) GO analysis of the up regulated genes after Ovol2-loss. (B) Expression analysis validation of key up regulated genes after Ovol2-loss.

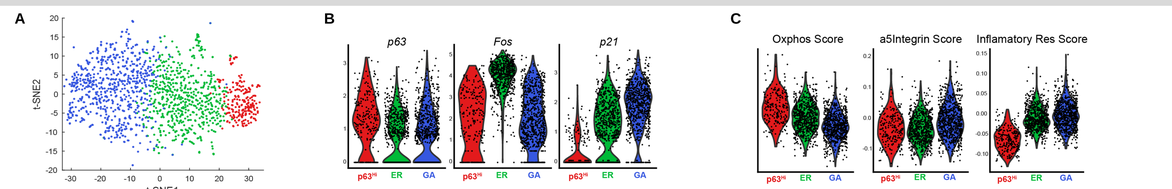

Figure 5: **Basal cell heterogeneity exists in wounded skin.** (A) tSNE plot of WO sample containing non-proliferative basal cells, which have unique marker genes. (B) Key markers in UW basal cell clusters (among others) are also seen in clusters of basal cells from WO sample indicating similar basal cell states exist in wound (note additional computational tools utilized to confirm). (C) Gene scoring for genes associated with oxidative phosphorylation, a5integrin/Cd51 (gene set associated with cells of the migrating front), and inflammatory response.

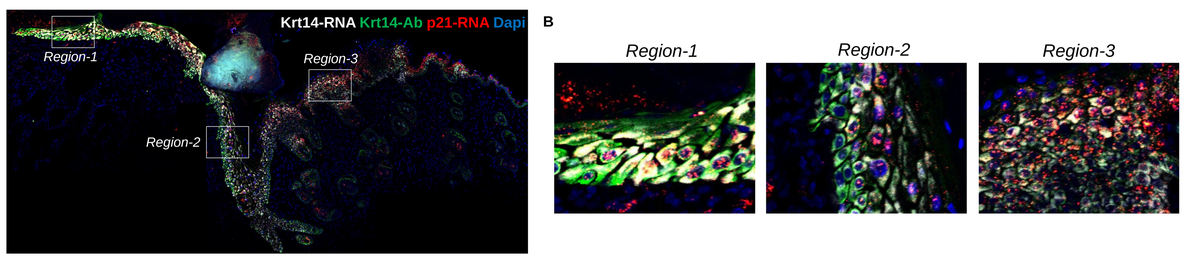

Figure 6: **In situ validation of basal cell heterogeneity in wounded skin by RNAScope.** (A) In situ analysis of marker genes using RNAScope in WO skin. Combinations of probes for RNA detection and antibodies for protein detected were utilized. (B) Zoomed in regions from (A). Region-1 has p21-positive basal cells indicating active cell cycle repression in highly migratory cells. Region-2 basal cells are p21-negative, with subrabasal cells solely being p21-positive.

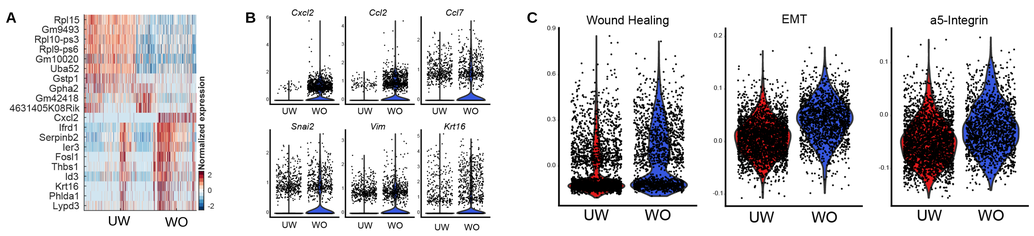

Figure 7: **Upregulation of EMT gene expression during wound healing.** (A) Heatmap comparing bulk basal cells from UW and WO samples. (B) Select genes upregulated in WO sample. (C) Gene scoring (score generated from group of genes that define a particular term or biological process) for Wound Healing, EMT, and a5-integrin (CD51, up-regulated in the migrating front of epidermal keratinocytes).

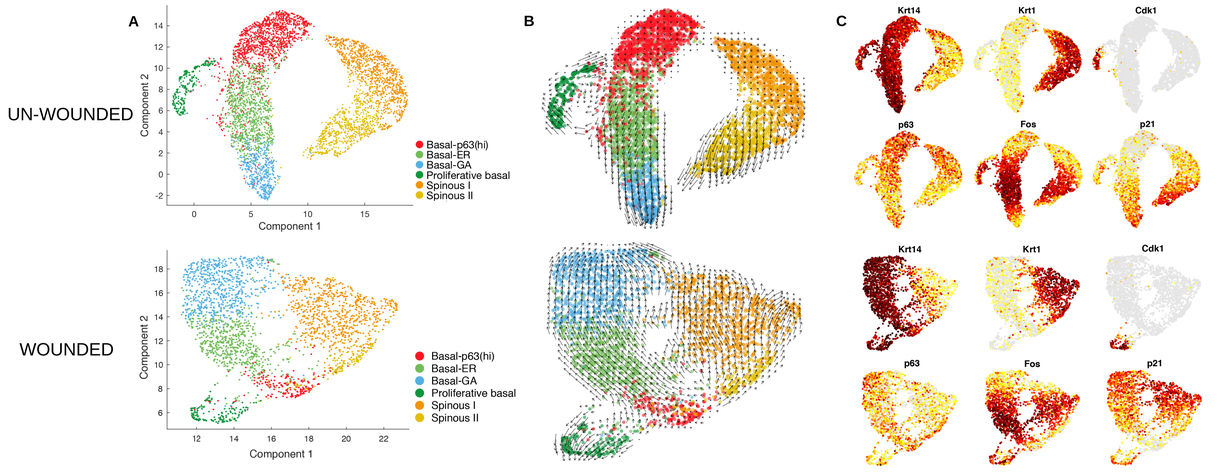

Figure 8: **Pseudotime and RNA velocity analyses of epidermal differentiation process in un-wounded and wounded skin.** (A) Cells, from un-wounded skin (upper) and wounded skin (bottom), were projected onto low-dimensional space generated by Uniform Manifold Approximation and Projection (UMAP), respectively. (B) RNA velocity analysis revealed two potential RNA velocity paths. (C) Feature plots of expression distribution for key marker genes. Cells with the highest expression level are colored dark red.

Multiscale Modeling of Epidermal Stratification in Cutaneous Wound Healing:

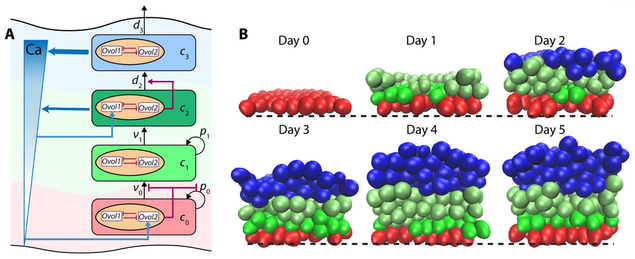

Figure 1: Preliminary multiscale modeling of epidermal stratification with inclusion of long-range morphogen regulation. (A) A schematic diagram shows the calcium regulation on Ovol. (B) Simulated time course images of layer formation.

- Ovol1 is expressed in suprabasal layers, in particular spinous layers, whereas Ovol2 is expressed predominantly in the basal layer of adult epidermis.
- Preliminary results suggest that long-range calcium signaling regulation enhances epidermis stratification and boundary formation.
- Future work is needed to study adult repair and regeneration in cutaneous wound healing to identify the similarities and differences in basic regulatory principles that govern epidermal development and homeostasis.

Figure 4: Loss of Zeb1 in Ovol2-defiencet cells rescues migratory phenotypes and directionality. (A) Adenovirus-Cre infection strategy and genotypes of cells used. Ovol2 KO cells have loss of only Ovol2 where DKO cells have loss of both Ovol2 and Zeb1. (B) Quantification of total distance traveled, velocity, and directionality of individual cells from colonies by live cell imaging.

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